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AN UNKNOWN TOXIC (OR ANTI-NUTRITIVE) SUBSTANCE IN THE SAGA-BEAN

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ABSTRACT

Biji saga (Adenanthera pavonina Linn), tergolong Leguminosae, dilaporkan dimakan oleh segolongan penduduk antara lain di daerah Pati (Jawa Tengah), sebagai makanan selingan "snack".

Penelitian menunjukkan bahwa kadar protein dan kwalitas protein biji saga terkupas hampir sama dengan protein biji kedelai. Kulit luar dan dalam biji saga merupakan kira-kira 55 % dari berat kotor, berbeda dengan kacang kedelai dengan angka antara 20 – 25 %.

Percobaan dengan anak tikus putih muda yang diberikan makanan mengandung biji saga menunjukkan adanya suatu "toxic factor". Berbeda dengan "toxic factor" yang juga ada pada kacang-kacangan lain, seperti kacang kedelai, "toxic factor" biji saga tidak dapat dihilangkan dengan perebusan dalam air selama 1½ jam. Keterangan lebih banyak mengenai sifat toxin ini belum diperoleh.

Perlu diadakan penelitian lebih lanjut untuk mendapatkan cara sederhana untuk menghilangkan zat toksik tersebut bila biji saga akan dipertimbangkan sebagai makanan manusia di pedesaan.

The saga tree (Adenanthera pavonina Linn.), also known as Bois de corail, Korallenbaum, Bead tree, Coral pea tree, belong to the family of leguminosae. The mature saga seeds, having a flaming red but very hard outer seed coat, are popular for making necklaces for children. As a food the saga bean was, according to Hasskarl (1845) as quoted by Heyne (1950), eaten as a sidedish with rice, after roasting and pounding for nulling, tasting like soy. Through the promotion of Soemartono (1977) the saga seeds have attracted the interest of policy-makers as a potential source of additional protein for the daily diet of the population. However, no information is available regarding its safety as human food.

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– This investigation was carried out in cooperation with the Asean Project on Soybean and Proteinrich foods (Indonesia).

MATERIAL.

Raw saga beans were obtained from Mr. Soemartono from the National Institute for Agricultural Research and Development, Min. of Agriculture, Pasar Minggu, Jakarta.

The beans were boiled in an open vessel with sufficient water for 1½ hour. The hard outer and inner seedcoat could then be removed by pressing and squeezing of each individual boiled bean between thumb and forefinger. After drying in the oven at 70° C the dehulled beans were ground to a course powder to make them suitable for the preparation of experimental diets.

METHODS.

a. Chemical analysis.

The proximate principles were determined according to the methods of A.O.A.C. (1975). The amino acid analysis, done according to the automated ion-exchange chromatography method, as described by Anderson and Jackson (1977), was carried out in the National Institute for Chemistry, Indonesian Institute of Sciences (LKN-LIPI), Bandung.

b. **Ratfeeding Experiments.**

All the ratfeeding experiments were carried out with young weanling albino rats of the Lembaga Makanan Rakyat (L.M.R.) strain in the Nutrition Unit Diponegoro-National Institute for Health Research and Development, Ministry of Health in Jakarta.

The composition of the experimental diets prepared are given in the ADDENDUM Tables I and 2.

RESULTS AND DISCUSSION.

Chemical Analysis.

– The result of the chemical analysis on the proximate principles of the sagabean as compared to soy is presented in Table 1 (See Table 1)

Table 1. Comparison of the proximate principles of Saga and Soy been (bolied, dehulled, dried)

(Average per 100 grams of) :		Saga	Soy
Moisture	%	4.8	5.3
Nitrogen	%	6.06	6.50
Protein (Nx 5.71)	%	34.6	37.1
Fat	%	18.0	20.0
Carbohydrate (by difference)	%	38.2	33.7
Fibre	%	12.6	4.0
Minerals	%	4.4	3.9
Refuse (saga outer and inner seedcoat)	%	55	20 – 25

– The outer and inner seedcoats, which are very difficult to remove in the raw state, consist of approx. 55% of the total weight of the bean, as compared to approx. 20 – 25% in the case of soybean. The protein and fat content of the dehulled beans are respectively 34 % protein and 18% fat for saga and 37% protein and 20% fat for soy.

The fibre content of the dehulled saga is very high, approx. 12%, while in soy it is approx. 4 % only.

The amino acid content of the saga and the soybean compared to the provisional amino acid pattern, are presented in Table 2.

Table 2. Essential amino acid content of Saga and Soy in mg per g Nitrogen (Compared to FAO/WHO provisional amino acid pattern).

	Saga mg	Soy mg	Provisional amino acid pattern FAO/WHO 1973 mg
1 Isoleucine	350	290	250
2 Leucine	600	494	440
3. Lysine	460	391	340
4. Methionine	60	84	–
5. Cystine	100	81	–
4 + 5. Total S-amino acids	(160)	(165)	(220)
6. Phenylalanine	437		–
7 Tyrosine	342	..	–
6 + 7 Total aromatic	(779)	(506)	(380)
8. Threonine	230	247	250
9. Tryptophan	–	76	60
10. Valine	385	291	310

The methionine content of the saga bean is somewhat lower than that of the soybean. But in both saga as well as soy, the total sulfurcontaining amino acids are the first limiting amino acids, a typical characteristic of legumes in general.

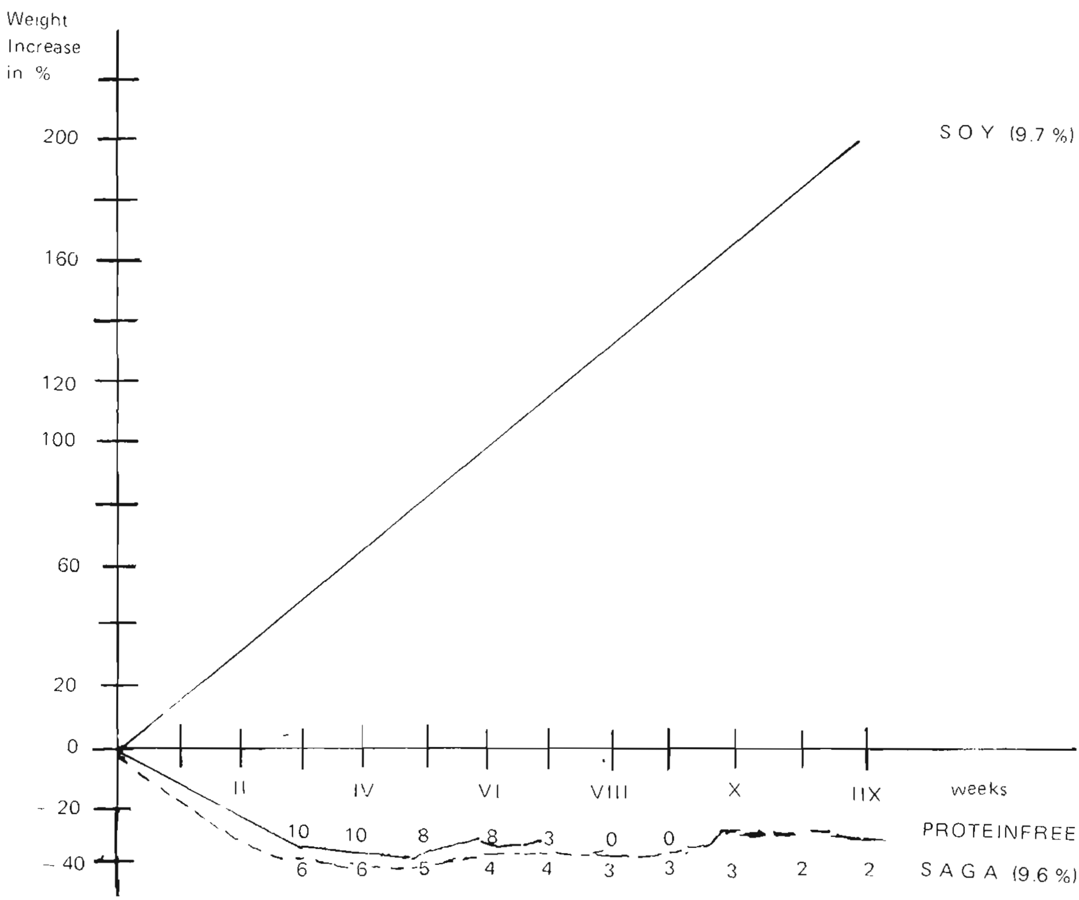
RATFEEDING EXPERIMENTS.

The growthcurves of the experimental rats fed 3 kinds of diets, viz. saga, soy and a proteinfree diet can be seen for Graph I. The first two experimental diets co... respectively 28 g of boiled dehulled dried saga and 27.8 g of boiled not dehulled soy per 100 g of diet. The remaining bulk consist of gelatinized starch which have no protein at all. The proteinfree diet consists mainly of this starch. Fat, vitamins and minerals are added in quantities according to recommended procedures to make the 3 diets similar in these aspects. See ADDENDUM table I for details.

It was expected that the saga diet (10% protein) should give the same good growth as the soy diet (10% protein). According to the chemical analysis the amino acid pattern of saga and soy are very similar (see Table 2) and yet, the results of the feeding trials were totally different. (See graph I)

The soy diet was well eaten and the growth of the rats was good. But the saga diet and also the proteinfree diet were refused by the rats. The very low food-intake was almost to the verge of total starvation (semi-starvation). This had led to a severe decrease of bodyweight causing ultimately marasmus and death, starting from the 5th week on, as seen in the groups of rats fed with the proteinfree as well as the saga diets. The instinctive refusal of an experimental diet by the rats is a good indication of something to be nutritionally wrong with the diet as fed. In the case of the proteinfree diet one may explain the instinctive refusal as a kind of regulating mechanism to reduce the body metabolic processes to the lowest minimum level as possible, due to the lack of protein in the diet.

Graph I. Growthcurves of young weanling albino rats fed a proteinfree diet and Saga and Soy diets both at 10% proteinlevel during 12 weeks.



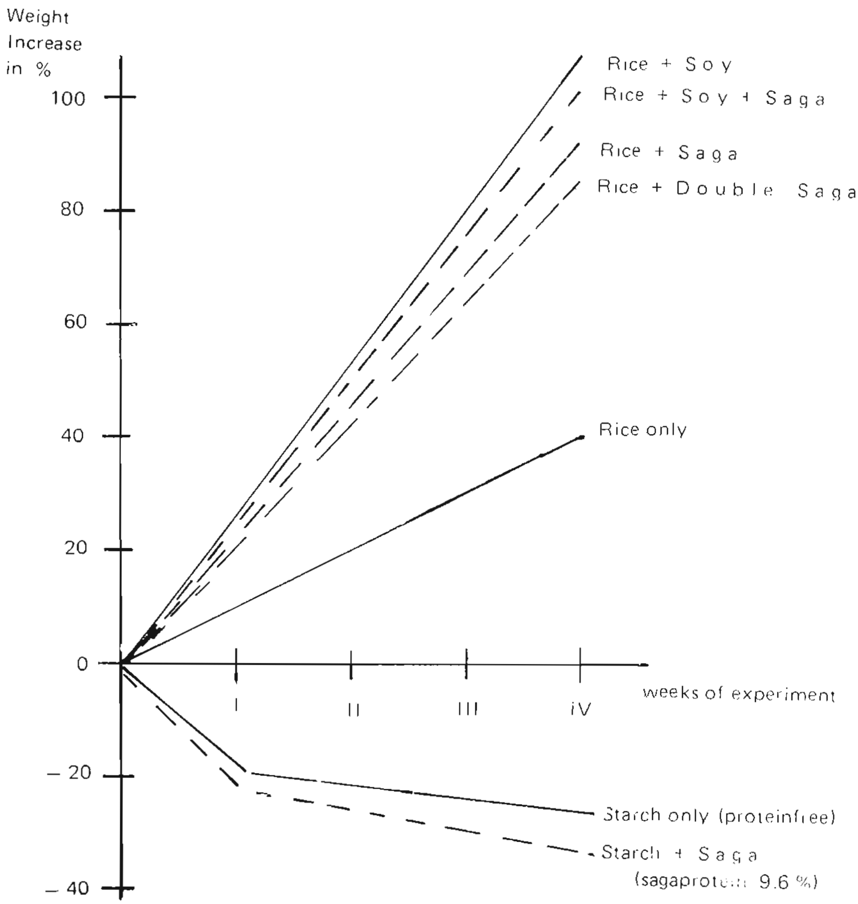
Note - Values within brackets connote the actual proteincontent of the respective experimental diets.
- Numbers show the numbers of experimental rats remaining alive during the week of experiment.
- For details of experiment, please see ADDENDUM Table V

A higher consumption of food would demand a higher metabolic activity. And as protein is missing, such an increase in metabolic activity would be very harmful to the rat.

It has to be mentioned that in the case of the proteinfree diet the reduced food intake and the negative growth rate (weight loss) are phenomena, familiar, constant and reproducible in ratfeeding experiments, where proteinfree diets were routinely fed as part of a biological evaluation of foodproteins.

But in the case of the 10% saga protein diet, this instinctive refusal cannot be due to the lack of protein. This saga diet contains 10% protein and the quality of the protein in this saga diet is also almost the same as the soy diet with 10% protein. One may think therefore of the presence of a harmful, toxic substance or of the lack of some essential amino acids through biological un-availability or of the persistence of the repellent beany flavour, all as the probable causes of refusal of this saga diet. In order to obtain more information, it is very important that the saga meal is indeed eaten in sufficient quantity by the experimental rats. Fortunately when the saga meal was incorporated into various diets of good protein quality, the saga was now well accepted by the rats (see ADDENDUM Table 2, 3) notwithstanding the persistence of the beany flavour in all the diets prepared. This can be seen from the results of the feeding trial with these experimental diets prepared from rice with saga and also with soy See Graph II

Graph II. Growthcurves of young weanling albino rats fed various rice diets mixed with Saga or Soy during 4 weeks.

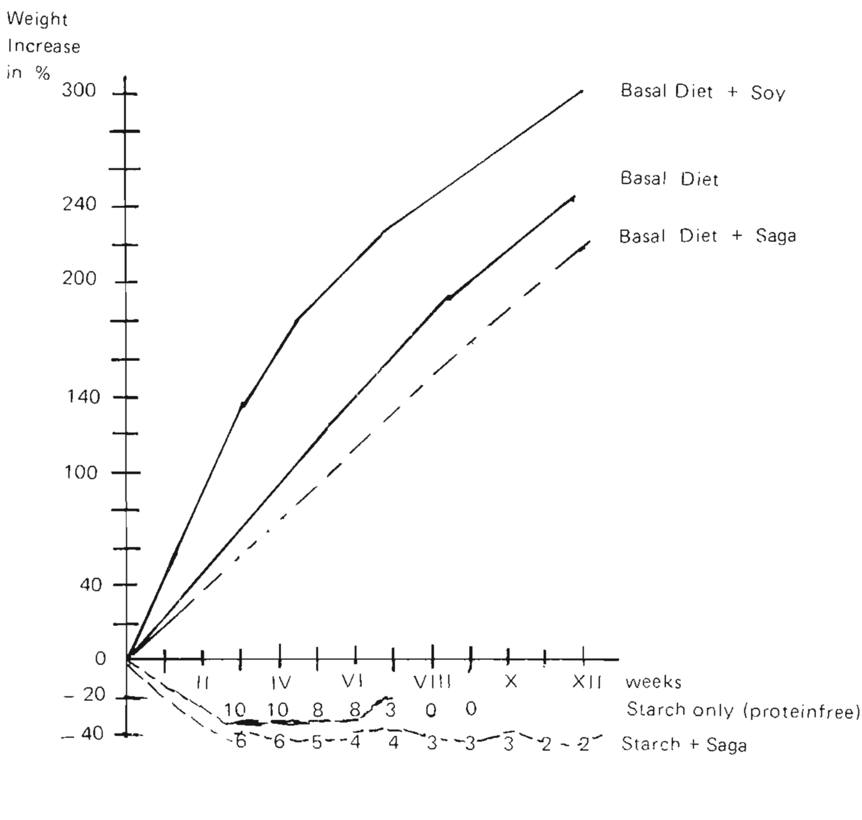


Note — For details of experiment, please see ADDENDUM Table IV

The saga proved to have a supplementary effect to rice, just like soy. This finding is a proof that all the essential amino acids must be biologically available and that the quality of saga protein is good. The persistent beany flavour apparently is not a major objection to the rats for consuming the diets in sufficient quantity during the whole period of experimentation. (See ADDENDUM Table 3 & Table 4). But another observation is the depression of the rates of growth of the young rats, when sagameal was added to otherwise good quality diets as can be seen from graph II. The growth rates of the rats on "rice + double saga" and "rice + soy + saga" were lower than the growth rates of the rats on "rice + saga" and "rice + soy" respectively. In fact, by adding saga, one should expect just like when adding soy, a higher rate of growth, as the saga protein is almost similar to soy in its amino acids content. (See Table 2). However the addition of saga to an otherwise good diet, has apparently a damaging effect, and this effect is probably related to the quantity of saga consumed (dose related). The total quantity of saga consumed during the 4 weeks of experimental period is presented in ADDENDUM Table 3. The growth depressing effect of boiled saga bean can be more conspicuously seen from graph III which depicts the results of a 12 week ratfeeding study.

This study is in fact equivalent to a subchronic toxicity test. A basal diet is used as a control. To this basal diet either soy or saga are added for comparison. The composition of the basal diet and the other 2 experimental diets can be seen from ADDENDUM Table 1.

Graph III. Growthcurves of young weanling albino rats fed a basal diet or mixtures with Soy or Saga during 12 weeks.



Note – For details of experiment, please see ADDENDUM Table V

When to this basal diet, soy was added, a pronounced increase in the growth rate was evident. But when a small amount of boiled saga was added to this same basal diet, the original growth rate became inhibited or depressed. This must be due to a toxic factor still present in the for 1½ hour boiled sagabeans.

In legumes, the growth inhibiting effect can be ascribed to the presence of protease (trypsin) inhibitors or phyto-hemagglutinins. As stated by Liener (1976), these substances can be eliminated by proper heat treatment. As the saga fed was subjected already to boiling for one-and-a-half hour, a boiling time considered more than usual, and yet still retaining the growth depressing effect, the substance very probably therefore may not belong tho the protease inhibitors or phytohemagglutinins as mentioned previously.

This preliminary study points out the need for an appropriate toxicity study which will throw more light on the toxic nature of the substance(s). Such an experiment should be carried out with a sufficient number of rats, at least 20 in each group, and the saga meal should be fed at 3 different dose levels.

The evaluation for toxicity must include the usual clinical observations, hematological and biochemical studies and also macroscopic and microscopic histopathologic examination of target organs (de Groot, A.P. 1974), all of which are procedures demanding facilities not yet properly available in our Unit. It is a general accepted rule, that the human species must be considered as the most sensitive species, when considering certain substances in food to be harmful or not. Based on the experimental findings presented above, the boiled saga bean proved to be harmful to rats and therefore also to human beings.

As a remark, one may wonder why the saga bean, so long known already by the population, until yet does not form a part of the cultural food pattern of the Javanese population, which is the case with the jengkol bean (Pithecolobium Lobatum) and the peteh beans (Parkia Speciosa), or the young pods of the lamtoro (Leucaena Glauca). But just like the toxic kapok seeds (Ceiba Pentandra) and the tamarind seeds (Tamarindus Indica), the saga bean was probably instinctively, by trial and error, avoided as a regular food by the population. Unless ways and means using grassroot technology applicable at the household level could be found to reduce the harmful substance(s) without affecting adversely the protein quality of the saga protein, the saga bean should officially not be recommended for use as a regulair daily human food. More studies by various disciplines are required to obtain a deeper insight on the potentials of the sagabean

CONCLUSION.

The protein of the saga bean is of good quality, having a good supplementary effect to rice protein, just like soy protein. Because of the presence of a toxic substance or antinutritive factor, which cannot be completely removed by boiling for 1½ hour, it is rejected by the young rats.

More investigation is needed on this toxic substance and its possible detrimental effect to human health. Ways and means should be found using socially acceptable grassroat technology to make it safe for human use, before any official recommendation could be issued for use as a daily food for the population.

ACKNOWLEDGMENT.

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Addendum Table 1 : Composition of Experimental Diets, as sed for growth Study on Rats Fed Saga lasting 12 weeks.
(See also graphs 1 and 3).

XP-diet designation		Protein free	Soy * 10 %	Saga * 10 %	Basal Diet (BD)	BD + Soy 10 %	BD + Saga 5 %
Nitrogen content	%	0	1.70	1.68	2.0	3.11	2.56
Protein content	%	0	9.7	9.6	11.9	18.5	15.2
Factor N (Nx ..)		0	(5.71)	(5.71)	(5.95)	(5.95)	(5.95)
Composition in g per kg XP-diet							
Starch		720	557	555	–	–	–
Fat to be added		100	50	50	–	–	–
Glucose		150	100	100	–	–	–
Salt mixture		20	15	15	–	–	–
Vitamin mixture		+	+	+	–	–	–
Celluflour		10	–	–	–	–	–
Basal Diet **		–	–	–	1000	722	860
Soy, boiled, not dehulled, dried		–	278	--	–	278	–
Saga, boiled, dehulled, dried		–	–	280	–	–	140
T o t a l		1000	1000	1000	1000	1000	1000

* Protein content of .
– Soy, boiled, not dehulled, dried 36.0 % (Nx 5.71) (N = 6.31 %)
– Saga, boiled, dehulled, dried 34.6 % (Nx 5.71) (N = 6.06 %)
** Composition of Basal Diet in grams per kg of diet

1	Rice	774 g
2.	Peanut	95 g
3.	Skimmilk powder	95 g
4	Coconut oil	17 g
5.	Salt mixture	13 g
6.	Bonemeal	6 g
7.	Vitamins: B-complex + A + D	+
8.	Iron sulfate	+
		± 1000 g

Protein conten ± 11.9 %
(Nx 5.95)

Addendum Table 2 : Composition of Experimental Saga-diets as prepared (See also Graph 2).

Components grams/per kg diet	Protein free	Saga 10 % protein	Rice alone	Rice + Soy	Rice + Saga	Rice + Double Saga	Rice + Soy + Saga
Fat added	100	50	90	33	33	26	26
Starch	720	555	–	–	–	255	235
Glucose	150	100	20	33	35	35	35
Salt mixture	20	15	10	10	10	10	10
Vitamin mixture	+	+	+	+	+	+	+
Cellu flour	10	–	–	–	–	–	–
Rice	–	–	880	866	868	566	582
Saga, boiled, dehulled, dried	–	280	–	–	54	108	54
Soy, boiled, not dehulled, dried	–	–	–	58	–	–	58
Total	1000	1000	1000	1000	1000	1000	1000
Results of Chemical Analysis of prepared dry diets							
Protein content g %	–	9.6	6.4	9.8	9.5	9.4	9.2
Nitrogen content g %	–	1.68 (Nx 5.71)	1.08 (Nx 5.95)	1.64 (Nx 5.95)	1.59 (Nx 5.95)	1.58 (Nx 5.95)	1.55 (Nx 5.95)
Fat g %	5 % – 6 % (except for the Proteinfree and Rice diets with 10 %)						

Addendum Table 3 : Summary of average total quantity of saga consumed per rat in 4 weeks XP-period.

Basal components of XP diets (numbers in italic connote grams of saga added per 100 g of diet)	Quality of Protein of original diet to which saga is added	Total Food Intake g	Avg. total quantity of saga Ingested during 4 week per-rat g	Body weight Increase % at end of 4th week g
Starch + Saga (28 g)	Protein free (starch only)	63.0	17.6	39.6
Rice + Saga (5.4 g)	Moderate (rice protein)	242.3	13.1	89.2
Rice + Double Saga (10.8 g)	Moderate (rice protein)	234.3	25.2	85.6
Rice + Soy + Saga (5.8 g + 5.4 g)	good (rice + Soy protein)	247.3	13.5	100.7
Basal diet + Saga (14 g)	Very good (Rice + Skimmilk + peanut protein)	223.2	31.5	116.7

Addendum Table 4 : Comparison of Average Body Weight Increase in % and average food intake in grams of young rats fed Saga or Soy mixed with rice by weeks.
(Feeding ad libitum For Growth curves see Graph no. 2)

	Description of XP–diet	No. of rats	Nitrogen Content	Protein Content	Protein distrib. Rice : Saga:Soy	Cumulative weight increase %				Avg. Food intake per rat by weeks				Avg. Total Food intake 4 weeks
						End of First week	End of Second week	End of Third week	End of Fourth week	I	II	III	IV	
		n	%	%	%	%	%	%	%	g	g	g	g	g
1.	Protein Free	10	0	0	–	–17.8	–24.6	–29.3	–32.8	20.0	19.9	17.2	16.7	73.8
2.	Soy, boiled not dehulled, dried (28.0 g soy/100 g)	10	1.70	9.7 (Nx5.71)	–	28.1	53.0	69.6	87.8	46.1	54.7	51.4	50.5	202.7
3.	Saga, boiled, dehulled, dried (27.8 g saga/100 g)	6	1.54	9.6 (N.x6.25)	–	–91.1	–29.4	–36.3	–39.6	17.9 s:5.0	17.4 4.9	14.5 4.1	13.2 3.7	63.0 s:17.6
4.	R i c e	10	1.08	6.4 (Nx5.95)	–	8.8	21.8	31.3	37.7	38.0	38.9	37.6	33.7	148.2
5.	Rice + soy (5.8 g soy/100 g)	10	1.64	9.8 (Nx5.95)	80: 0:20	29.5	58.1	79.7	104.0	58.1	68.4	64.8	62.2	253.5
6.	Rice + saga (5.4 g saga/100 g)	10	1.59	9.5 (Nx5.95)	80:20: 0	23.8	49.2	68.8	89.2	55.3 s:3.0	64.2 3.5	62.4 3.4	60.4 3.3	242.3 s:13.1
7.	Rice + double saga (10.8 g saga/100 g)	10	1.58	9.4 (Nx5.95)	55:45: 0	24.1	45.4	65.8	85.6	54.7 s:5.9	63.4 6.8	59.3 6.4	56.9 6.1	234.3 s:25.2
8.	Rice + soy + saga (5.8 g soy and 5.4 g saga/100 g)	10	1.55	9.2 (Nx5.95)	55:22:23	28.6	51.8	77.9	100.7	57.5 s:3.1	65.1 3.5	61.5 3.3	63.2 3.4	247.3 s:13.5

s = Avg. intake of saga per rat in grams.

Average weight of rats at the start of the experiment is 60.1 grams except for "rice + soy + saga" 47.9 grams.

Addendum Table 5 : Comparison of Average Cumulative Body Weight Increase of young rats fed Saga or Soy or incorporated in Basal Diets by weeks in %
(Feeding ad libitum) (For Growth Curves see Graphs no. 1 and 3)

	Description of XP–diet	Prot. Cont.	Avg. Cumulative Weight Increase in % of starting weight at End of Week												Avg. Total Food Intake 12 weeks
			I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	
		%	%	%	%	%	%	%	%	%	%	%	%	%	gram
1.	Protein-free diet	0	–12.3	–18.9	–27.5	–27.5	–32.6 n=8	–23.6 n=8	–20.8 n=3	– n=0	–	–	–	–	–
2.	Soy, boiled, not dehulled, dried 27.8 g/100 g	9.7	28.1	53.0	69.6	87.8	108.7	128.4	145.5	154.9	168.3	181.8	186.5	193.3	202.7
3.	Saga, boiled, dehulled, dried 28.0 g/100 g.	9.6	–19.1	–29.4	–36.3	–39.6	–41.8 n=5	–36.3 n=4	–35.7 n=4	–36.9 n=3	–39.9 n=3	–33.8 n=3	–39.5 n=2	–38.2 n=2	–
4.	Basal diet	11.9	31.2	71.2	100.5	129.0	155.1	173.9	195.0	208.1	220.1	220.4	235.3	249.3	210.4
5.	Basal Diet + Soy 27.8 g/100 g.	18.5	51.9	97.4	128.1	156.4	186.0	209.2	230.9	243.7	256.3	272.6	280.7	283.3	233.0
6.	Basal Diet + Saga 14.0 g/100 g.	15.2	35.3	61.0	89.8	116.7	137.3	186.5	171.0	177.4	187.4	199.7	196.6	213.5	223.2

= Average weight of rat at start of experiment : approx. 48.0 grams.

= Number of rats per XP–diet : n = 6. (except diet 1 & 2, n = 10).